

**Appl. No.** : 10/628,879  
**Filed** : July 28, 2003

### **INTERVIEW SUMMARY**

Applicants thank Examiner Yang for the helpful interviews conducted on September 27 and October 4, 2005 with Eli Loots. During the interviews it was generally agreed that the teachings in the French reference do not teach or suggest analyte detection via anisotropy where a fluorophore associated with a receptor (e.g., an aptamer) is attached to a surface before the detection or illumination step. Instead, French teaches sample detection upon binding of a fluorophore to a surface. It was noted that in French it is the change in anisotropy due to the change in mobility of the fluorophore in solution compared to mobility on the surface that is detected. In contrast, in the present claims the fluorophore-labeled aptamer is bound to the surface both before and after; thus, the change in anisotropy is not due to a change as taught in the French reference. The possibility of an amendment to Claim 1 to reorganize the recited elements in item (a) was also discussed. In addition, the Examiner provided the Applicants' Representative with an additional reference, Terpetschnig et al. (2001/0018194) for possible comment in the present Response. Additional aspects of the interview are discussed below.

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### REMARKS

Claims 1-21 are pending and stand rejected on a variety of grounds. Claim 1 has been amended to recite "contacting a sample with a fluorophore-labeled aptamer bound to a solid support..." Support for this amendment can be found throughout the Claims and the specification, for example, in original Claim 1. The amendment adds no new matter and simply reorders the elements to clarify the relationship between the fluorophore-labeled aptamer, the solid support, and the sample.

Applicants thank the Examiner for the review of the previous amendments and remarks and the subsequent withdrawal of the rejections regarding Potyrailo, Fang, Stanton, Lackowicz, Gold, and Lee.

#### **Rejections under 35 U.S.C. §103(a) over French in light of Lee**

The Examiner has rejected Claims 1-9, 11-19, and 21 under 35 U.S.C. §103 as being obvious in view of the combination of French (U.S. Pat. No. 6,297,018) and Lee ("A fiber-optic microarray biosensor using aptamers as receptors," 2000, *Anal Biochem.*, 282:142-146, hereinafter "Lee").

French has been asserted as teaching assays for detecting nucleic acid targets with primers using polarization of luminophores. Furthermore, French was found to teach the attachment of the components to a solid support, such as a mass label or a solid surface. French does not teach aptamers. It has been asserted that Lee teaches silica beads and fluorescent measurements. The Examiner found that the motivation for combining these references is that the combination would provide an assay for disease related protein detection that is quick, sensitive, convenient, and selective. The Applicants respectfully traverse.

As discussed in the Interview, in present Claim 1 the fluorophore-labeled aptamer is bound to a solid support when the sample is contacted with it. In the method recited in Claim 1, the change in anisotropy is from 1) a fluorophore that is attached to the aptamer, where the aptamer is on a solid support to 2) a fluorophore that is attached to the aptamer, which is bound to the analyte, attached to a solid support. Thus, the fluorophore is attached to a surface both before and after the detection or measured change in anisotropy upon analyte binding. In contrast, French and many of the previously cited references teach that it is the binding or association of the fluorophore to the solid support that results in the change in anisotropy and

thus the detectable signal. One reason these references teach such a method is the large change in mass between 1) the fluorophore in solution (associated with relatively small protein of interest) and 2) the fluorophore (associated with a protein of interest) associated with a support surface. As the change in mass of the complex associated with the fluorophore is large between step 1 and 2, association with the support, according to their theory, results in a large change in mobility, which results in a large change in anisotropy. This is shown in French on col. 8, lines 52-64, which indicates that the mobility of the unlabeled polynucleotide can be reduced by attaching it to a surface or through the use of mass labels. Figure 2 further demonstrates that the change in anisotropy detected occurs from the fluorophore (ddNTP) going from solution to being associated with a surface (thereby reducing the mobility of the fluorophore). This is also discussed in col. 5, lines 1-3, col. 8, lines 13-27, and col. 9, lines 7-10 of French. Thus, French does not teach or suggest a sample with a fluorophore labeled aptamer bound to a solid support as recited in Claim 1. Applicants note that the other steps in Claim 1 (*e.g.*, direct illumination when using a surface associated probe) are either not likely to be the same as the methods in French because step a) is different, or are not clearly taught in French in light of the previously cited teachings (in the prior rejections) which suggested particular forms of illumination.

Finally, French teaches that, at the detection event, one should have a large percent change in mass, allowing for a large change in mobility, and thus a large change in anisotropy. To achieve this, one initially has the fluorophore (and what it is associated with) in solution. In contrast, the currently recited probes are already attached to a large surface, giving them, under the eyes of French, a large initial mass. Because of this, the binding event involves a much smaller percent change in mass than if the fluorophores were initially in solution and then bound to a larger surface. Thus, while French and the other references teach that the percent change in mass should be optimized, if not maximized, for the detection event, the presently claimed invention involves a smaller percent change in mass upon binding because the aptamer is associated with the surface both before and after the detection event. As such, French and the other references teach away from the presently claimed method.

Applicants note that the secondary reference, Lee, is not directed to anisotropy and does not provide the required teachings regarding Claim 1. As such, neither of the references, nor their combination, makes up for the above identified deficiencies. Lakowicz and Gold, cited in the rejections of Claims 10 and 20, also do not supply the missing elements noted above.

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As not all of the elements have been taught by the cited references and there is no motivation for modifying the references, a *prima facie* case of obviousness has not been established. Applicants respectfully request that the Examiner withdraw the rejection and allow the claims. Even if such a case had been established, it would be rebutted by the fact that many of the references teach away from the claimed invention. Furthermore, as Claims 2-21 depend from Claim 1, and Claim 1 is novel and nonobvious, Applicants note that Claims 2-21 must also be novel and nonobvious. Additionally, Applicants note that the dependent claims recite further novel and nonobvious aspects and combinations.

In addition to the French reference, in the interview the Examiner discussed the relevance of Terpetschnig et al. (U.S. Pat. Pub. No: 2001/0018194), and in particular, paragraphs 0052-0064 and the discussion on polarization assays a paragraphs 0065-0069. As an initial point, Applicants note that the use of "particulates" in Terpetschnig is assay specific; thus, not all of the discussion of particulates necessarily applies to every form of polarization assay or resonance energy transfer assay (see, *e.g.*, paragraph 0060). As such, the entirety of the reference must be considered in the analysis (especially paragraphs 0028-0037 and 0070-0092).

In the interest of accelerating prosecution, Applicants note that there are differences between the particulates and methods in Terpetschnig and the claimed methods. As a general summary of Terpetschnig, fluorophores are preferably immobilized in a particulate so that mobility of the particulate functions as an indicator of fluorophore freedom. Associated with the particulate is a receptor molecule; however, in Terpetschnig the fluorophore is not bound to the receptor molecule. Rather, the fluorophores are associated with the particulate and the receptor molecule is separately associated with the particulate. One then uses the entire particulate in a manner similar to that used in French. For example, one looks for binding of the particulate (and all of the fluorophores associated with it), via, *e.g.* a ligand, to a receptor on a membrane. As in French, the mobility of the fluorophores with the particulate goes from relatively high as the entire particulate rotates in solution, to relatively restricted, as it binds to, *e.g.*, a receptor on a membrane. As such, some of the above arguments regarding French are also relevant here.

An additional difference between the presently claimed invention and the teachings of Terpetschnig is that the fluorophores in Terpetschnig are directly associated with the particulate. Preferably, the fluorophores are immobilized in the particle when the particle is to be used for a

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polarization assay (e.g., paragraphs 0054 and 0067).<sup>1</sup> In contrast, Claim 1 recites a fluorophore labeled aptamer. Applicants note that the interaction between the aptamer, the analyte, and the fluorophore is what the Applicants theorize induces the detectable change in anisotropy. Applicants note that such an arrangement and the resulting change are not present in *Terpetschnig*. In fact, *Terpetschnig* actually teaches away from such a combination. For example, *Terpetschnig* teaches that the direct association of the fluorophore with a ligand (in the Applicants' case the aptamer) is an undesired combination as it increases variability and can interfere with binding of the ligand to an analyte.<sup>2</sup> Thus, the present claims are not anticipated by or made obvious in light of *Terpetschnig*.

### Conclusion

Applicants respectfully submit that for the above-recited reasons the rejections should be withdrawn and the claims allowed. If, however, some issue remains, the Examiner is cordially invited to telephone the undersigned in order to resolve such issue promptly. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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<sup>1</sup> This is done so that "depolarization reflects reorientation of the labeled molecule and not merely reorientation of the label relative to the labeled molecule." (paragraph 0067).

<sup>2</sup> See, e.g., paragraph 0011, "For example... the label may interfere with the biological activity being assayed, or the label may alter the solubility of the compound being labeled, causing it to precipitate." and "[i]ncorporation of the label in a particulate may reduce the variability that results from attaching the label directly to the molecule of interest." (paragraph 0055).